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Apiosordaria hamata sp. nov. from lake sediment in China

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Abstract—A new species, *Apiosordaria hamata*, is described and illustrated from sediment collected from Donghu Lake, Wuhan, Hubei Province, China. *Apiosordaria hamata* is characterized by its cleistothecial ascomata with agglutinated hairs, a thin peridium, clavate asci, and two-celled, biseriate, ovoid ascospores. However, it differs from other *Apiosordaria* species in having hooked spines on the upper cell of the ascospores and lacking germ pores. Combined ITS and LSU sequence analyses using maximum likelihood and Bayesian analysis nest *A. hamata* in the *Apiosordaria* clade. Both morphological and molecular analyses support this fungus as a new species of *Apiosordaria*. A key to *Apiosordaria* species is provided.

Key words—phylogenetic analyses, soil fungi, *Lasiosphaeriaceae*, *Sordariales*, taxonomy

Introduction

Arx & Gams (1967) introduced the genus *Apiosordaria* Arx & W. Gams (*Lasiosphaeriaceae*, *Sordariales*) based on the type species *Pleurage verruculosa* C.N. Jensen [≡ *A. verruculosa*]. Krug et al. (1983) synonymised *Echinopodospora* and *Lacunospora* under *Apiosordaria* based on morphological similarities. In *Apiosordaria* ascospores are usually two-celled, composed of a dark upper cell and a lower hyaline cell; the upper cell is ellipsoidal to subglobose, with walls ornamented with numerous broad or narrow pits; the lower cell is triangular to cylindrical and possessing a minute hyaline terminal appendage (Arx & Gams 1967). In addition, the presence of spines, warts, or pits on the upper ascospore cell is used as a principal character that differentiates this genus from related

genera in the *Lasiosphaeriaceae* that also produce two-celled ascospores (e.g., *Cercophora*, *Podospora*, *Triangularia*, *Zopfiella*; Garcia et al. 2003).

Apiosordaria currently comprises 23 species with a worldwide distribution in garden soils (Stchigel et al. 2000), field soils (Jensen 1912), grassland soils (Warcup 1951a), and forest soils (Warcup 1951b). The genus is also found in dung (Krug et al. 1983) and decaying wood (Badura & Badurowa 1964). Some species have also been found associated with plant material (Rostrup et al. 1916) and the insect *Plecia nearctica* (Kish et al. 1974).

During a study of the fungal diversity of wetlands in China, an interesting *Apiosordaria* species was isolated. Morphological characteristics distinguishing this taxon from all other species in the genus (Krug et al. 1983, Guarro & Cano 1988, Stchigel et al. 2000) are here supported by phylogenetic analyses. In this paper, the new species is introduced, described, and illustrated, and compared with the most similar species.

Materials & methods

Collection, isolation, and morphological examination

Soil samples were collected from Donghu Lake (30°33′N 114°23′E) in the northeastern outskirts of Wuchang, Wuhan City, Hubei, China. Donghu Lake, situated on the alluvial plain in the middle basin of the Changjiang (Yangtze) River, has a total surface area of 32 km^2 and lies 21.4 m (20.8–21.7 m) above sea level. The lake is usually 3–4 m deep with a maximum of 4.5 m, and the flat muddy lake bottom is sparsely covered with hydrophytes. The area is dominated by a damp subtropical monsoon climate, with a temperature range of 12.9–21.5 °C and annual precipitation of ca. 1040 mm. Sediment samples were collected along the bank of the lake at depths of 0–40 cm. The samples were sealed in plastic bags, placed in a 4 °C car refrigerator, and transported to the laboratory for processing.

A 10 g sediment subsample was homogenized, serially diluted in sterile distilled water, shaken, and 200 µl plated onto ¼ strength potato dextrose agar (PDA) containing 100 mg l^{-1} streptomycin and 100 mg l^{-1} chloromycetin. The plates were kept at 25 °C for 1 week, after which fungi with different colony morphologies were selected and grown on new PDA plates with antibiotics, on oatmeal agar (OA), potato carrot agar (PCA, potato 20 g, carrot 20 g, agar 20 g) and malt extract agar (MEA). Isolates were identified based on morphological characteristics after 10 days incubation at 25 °C.

Microscopic characteristics of the new species of *Apiosordaria* were observed with a Nikon Ellipse 80i light microscope equipped with differential interference contrast (DIC) optics and a FEI Quanta 200 scanning electron microscope (SEM). Ex-type and isotype cultures are deposited in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China.

DNA sequencing & phylogenetic analysis

After 10 days growth on PDA, ca. 0.2 g of mycelium was collected with a sterile scalpel and placed into a 1.5 ml centrifuge tube. Genomic DNA was extracted with

Table 1. Sources and sequences used in the phylogenetic analysis.

Taxon	Collection no.*	Origin	GenBank accession no.	
			ITS	LSU
Apiosordaria backusii [≡ *Triangularia backusii*]	CBS 106.77	Sandy soil, Japan	GQ922520	—
A. hamata	CGMCC 3.15230	Wetland soil, China	**KP878306**	**KP878304**
A. hamata	CGMCC 3.15231	Wetland soil, China	**KP878307**	**KP878305**
A. longicaudata	CBS390.84	Soil, Japan	—	FR692340
A. nigeriensis	FMR 6363	Garden soil, Nigeria	AJ458184	—
A. tetraspora	CBS 363.84	Soil, Thailand	—	FR692341
A. verruculosa	F-152365	Ethanol-pasteurized soil, Spain	—	AY346258
A. verruculosa var. *maritima*	CBS 550.66	Ethanol-pasteurized soil, Spain	—	FR692345
Apodus deciduus	CBS 506.70	—	AY681199	AY681165
A. oryzae	CBS 376.74	—	AY681200	AY681166
Bombardia bombarda	AFTOL 967	—	—	DQ470970
Bombardioidea anartia	HHB99-1	Moose dung	—	AY346264
Cercophora areolata	UAMH7495	—	AY587911	AY587936
C. sparsa	JF00229	—	—	AY587937
C. sulphurella	SMH2531	—	AY587913	AY587938
Cladorrhinum sp.	MJ-2014	—	KJ572182	—
Immersiella caudata	SMH3298	—	—	AY436407
I. immersa	SMH2589	—	—	AY436408
Jugulospora rotula	ATCC38359	Branch, Panama	—	AY346287
Lasiosphaeria glabrata	TL4529	—	AY587914	AY436410
L. lanuginosa	SMH3277	—	AY587920	AY587943
L. ovina	CBS 958.72	Decaying wood, Germany	AY587925	AY587946
L. rugulosa	SMH4438		AY587932	AY587952
Podospora cupiformis	CBS 246.71	*Kobus defassa* dung	AY999125	AY999102
P. curvicolla	IFO 8548	Rabbit dung	AY999122	AY999099
P. intestinacea	CBS 113106	Horse dung	AY999121	AY999104
Schizothecium aloides	CBS 879.72	Soil, Netherlands	AY999120	AY999097
S. fimbriatum	CBS 144.54	Horse dung	AY999115	AY999092
S. glutinans	CBS 134.83	*Arctostaphylos uva-ursi*, Switzerland	AY999116	AY999093
Strattonia carbonaria	ATCC 34567	Burned soil, Japan	—	AY346302
Triangularia mangenotii	ATCC 38847	Soil, Japan	—	AY346303
T. tanzaniensis	TRTC51981	—	—	AY780081
Xylaria hypoxylon	AFTOL-ID 51	Downed rotting wood	DQ491487	AY544648
Zopfiella tabulata	CBS 230.78	Porcupine dung, Canada	AY999132	AY999105
Zygopleurage zygospora	SMH4219	Dung of cow, USA	—	AY346306

* ATCC, American Type Culture Collection, USA; AFTOL, Assembling the Fungal Tree of Life, USA; CBS, Centraalbureau voor Schimmelcultures, Netherlands; CGMCC, China General Microbiological Culture Collection Center, China; HHB, Field Museum of Natural History, Chicago, Illinois; IFO, Institute for Fermentation, Osaka; SMH, Sabine M. Huhndorf; TL, Thomas Læssøe; UAMH, University of Alberta Microfungus Collection and Herbarium.

a simple and rapid 'thermolysis' method (Zhang et al. 2010). Partial 28S rDNA and complete ITS+5.8S rDNA were amplified using fungal specific primers LROR/LR7 (Vilgalys & Hester 1990) and ITS5/ITS4 (White et al. 1990), respectively.

All PCR amplifications were carried out using a Biometra T-professional thermocycler. The reaction mixture included 5 µl of 10× Takara Bio PCR buffer, 2 µl of 25 mM $MgCl_2$ (1 µM final concentration), 4 µl of 2.5 mM deoxynucleotide triphosphate mix (final concentration, 250 µM each; Takara Bio, Japan), 2 µl of 10 µM primers each (final concentration, 0.25 µM; Sangon Biotech, China), 1 µl Sigma deionized formamide, and 0.4 µl Taq polymerase. The amplification was carried out as follows: 95 °C for 3 min; followed by 35 cycles at 95 °C for 1 min, 54 °C for 45 s, and 72 °C for 90 s; and a final extension for 10 min at 72 °C. After the PCR products were separated in 1% (w/v) agarose gels, they were stained with 0.4% GoldView nucleic acid gel stain (SBS Genetech) and viewed and photographed on a Gel imaging system (Bio-Rad).

The internal transcribed spacer (ITS) region and large-subunit rRNA (LSU) gene data sets were constructed with sequences of *Apiosordaria hamata* (isolates CGMCC 3.15230, CGMCC 3.15231) and reference sequences from related taxa retrieved from GenBank (Table 1). All sequences were aligned with ClustalX v1.83 (Thompson et al. 1997), and the alignment was manually adjusted to allow maximum alignment and minimize gaps. Maximum likelihood (ML) and Bayesian analysis were used to estimate phylogenetic relationships of *A. hamata* with its related taxa, with *Xylaria hypoxylon* (L.) Grev. as the outgroup taxon.

Maximum Likelihood analysis was conducted in RaxmlGUI v1.3 (Silvestro & Michalak 2012) with the GTRGAMMAI model, 1000 bootstrap replicates. While Bayesian analysis was performed in a likelihood framework as implemented by MrBayes v3.0b4 software package to reconstruct phylogenetic trees (Huelsenbeck & Ronquist 2001), the best-fit evolutionary model was determined by MrModeltest v2.3 (Posada & Crandall 1998, Nylander 2004) through comparing different nested DNA substitution models in a hierarchical hypothesis-testing framework. The best-fit model determined by MrModeltest was the "GTR+I+G", lsetnst = 6, rates = invgamma; prset statefreqpr = dirichlet (1, 1, 1, 1) for Bayesian analyses. Multiple Bayesian searches using Metropolis-coupled Markov chain Monte Carlo sampling were conducted using one cold and three heated Markov chains in the analysis. Bayesian analysis was run for 645,000 generations, with trees sampled every 100 generations. The first 1290 trees, representing the burn-in phase, were discarded. To estimate posterior probabilities (PP) of recovered branches (Larget & Simon 1999) 50% majority rule consensus trees were created from the remaining trees using PAUP.

Results

Molecular phylogeny

Nucleotide sequence blasts of the NCBI-nrDNA database showed that the new fungus, *Apiosordaria hamata*, exhibited 98% LSU sequence similarity with *Cercophora* sp. isolate SMH3200 (AY780055) (query cover = 99%, identities = 1274/1295, gaps = 2/1295), 97% similarity with *Arnium arizonense* isolate 18211-c (S) (KF557668) (query cover = 100%, identities = 1252/1297, gaps

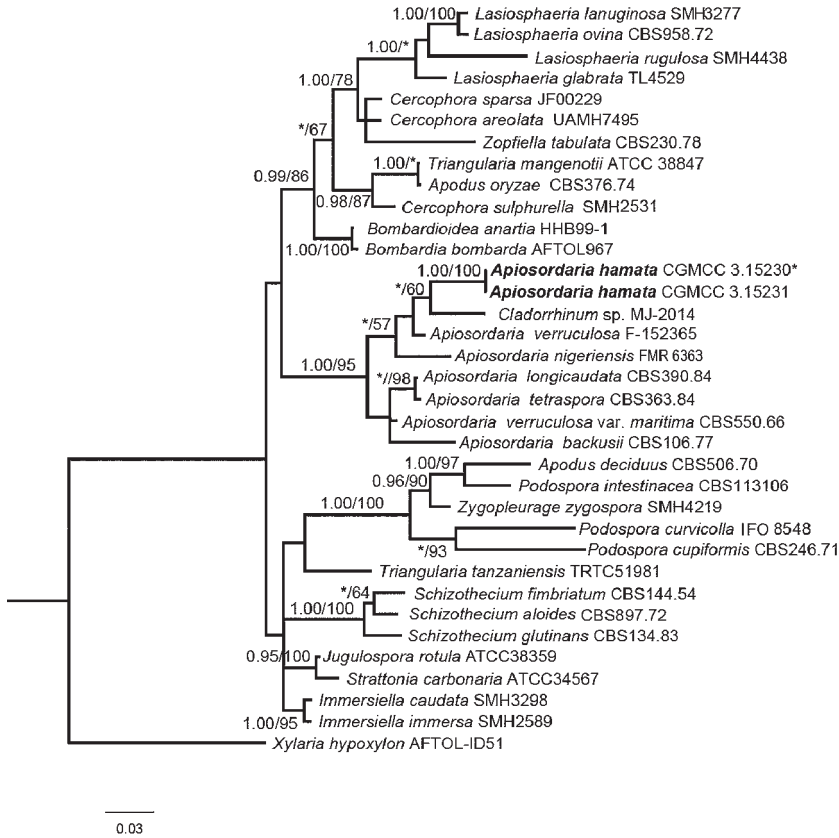


Fig. 1. Maximum likelihood (ML) tree from PAUP analysis and Bayesian tree by MrBayes based on LSU and ITS sequences of *Apiosordaria* and GenBank data from related species. Bayesian posterior probabilities >0.95 and ML bootstrap support values > 50% are shown on the upper branches.

= 3/1297), and 97% similarity with *Arnium arizonense* isolate 724 (UPS) (KF557669) (query cover = 99%, identities = 1250/1295, gaps = 3/1295) in family *Lasiosphaeriaceae*. ITS sequence results indicated a 92% similarity with *Cladorrhinum* sp. MJ-2014 (KJ572182) (*Lasiosphaeriaceae*) (query cover = 100%, identities = 498/544, gaps = 10/544). We therefore included related reference sequences in *Lasiosphaeriaceae* in our phylogenetic analyses.

The LSU and ITS combined gene datasets were analyzed using maximum likelihood (ML) and Bayesian analysis (Fig. 1). The phylogenetic trees derived from ML and Bayesian analysis gave similar results; the *Apiosordaria hamata*

strains (CGMCC 3.15230 and CGMCC 3.15231) formed a well-supported clade with *A. backusii* (L.H. Huang) Guarro [≡ *Triangularia backusii*] (CBS 106.77), *A. longicaudata* (Furuya & Udagawa) J.C. Krug et al. (CBS390.84), *A. nigeriensis* Stchigel & Guarro (FMR 6363), *A. tetraspora* J.C. Krug et al. (CBS 363.84), *A. verruculosa* (C.N. Jensen) Arx & W. Gams (F-152365), *A. verruculosa* var. *maritima* (Apinis & Chesters) Arx & W. Gams (CBS 550.66), and *Cladorrhinum* sp. (MJ-2014) within *Lasiosphaeriaceae* (Bayesian posterior probability = 1; ML bootstrap value = 95%). However, the two *A. hamata* strains formed a separate clade with high support (Bayesian posterior probability = 1; ML bootstrap value = 100%).

Taxonomy

Apiosordaria hamata B. Wu, K.D. Hyde, Jing Z. Sun & Xing Z. Liu, **sp. nov.** Fig. 2

MycoBank MB 812280

Differs from related *Apiosordaria* species by lacking germ pores in the ascospores and by having hooked spines on the upper cell of the ascospores.

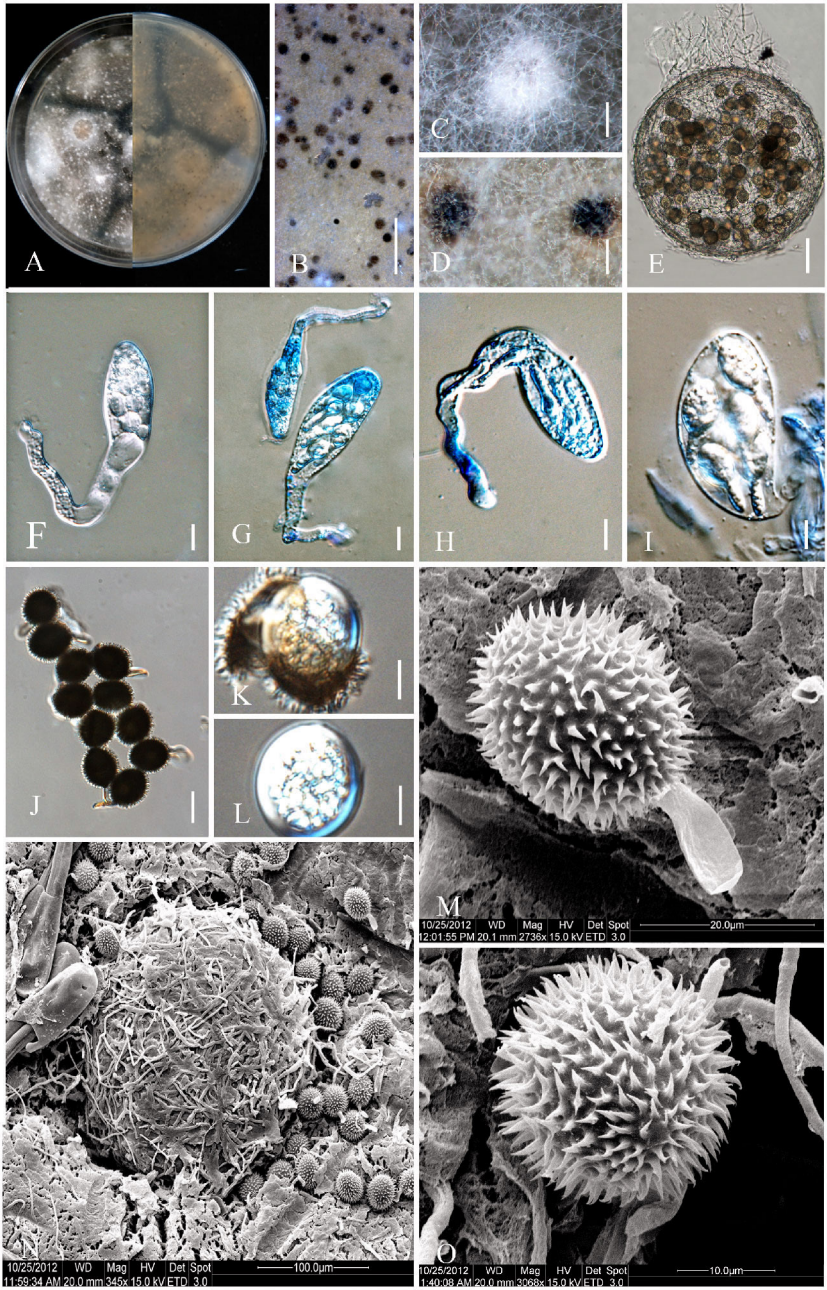
Type: China, Hubei, Wuhan, Donghu Lake, isolated from sediment, July 2009, B. Wu (**Holotype**, HMAS 246231; ex-type cultures, CGMCC 3.15230, CGMCC 3.15231).

Etymology: *hamata* refers to the hooked spines on the upper cell of the ascospores.

Faces of Fungi number: FoF 00663

Ascomata superficial, scattered, cleistothecial, dark brown, globose, overall 475–490 × 480–525 µm. Peridium 4–6-layered, up to 4–5 µm wide, translucent, brownish orange to brown, comprising cells arranged in a textura angularis. Paraphyses hyaline, filiform, septate, unbranched, 1.8–2.2 µm wide. Asci 8-spored, clavate, broadly rounded at the apex, 420–450 × 90–100 µm, pedicel without conspicuous apical structures, evanescent, 90–150 µm long. Ascospores biseriate, clavate, hyaline, 1-celled when young and becoming two-celled with the formation of a transverse septum, warted wall, 26–31 × 22–26 µm; upper cell obovoid, truncate at the base and with a slightly acuminate apex, brown, thick-walled, uniformly ornamented with numerous 2–3 µm diam (x = 2.4 µm, n = 30) hooked spines, lacking a germ pore; lower cell sub-hyaline, conical and smooth, 4–7 × 12–15 µm (x = 5.2 × 13.1 µm, n = 30) long. Asexual morph: Undetermined.

Fig. 2. *Apiosordaria hamata* (holotype, HMAS 246231). A. colony on PCA; B, ascomata; C. immature ascoma; D, E. mature ascomata; F. immature ascus; G–I. mature asci in cotton blue; J. ascospores; K. ascospore with warted coat; L. ascospore with smooth coat; M, O. ascospores with hooked spines (SEM); N. ascoma (SEM). Scale bars: B = 1 mm; C, D = 100 µm; E = 50 µm; F–I, K, L = 10 µm; J = 20 µm.


A
B
C
D
E
F
G
H
I
J
K
L
M
10/25/2012 WD Mag HV Det Spot
12:01:55 PM 20.1 mm 2736x 15.0 kV ETD 3.0
20.0µm
N
10/25/2012 WD Mag HV Det Spot
11:59:34 AM 20.0 mm 345x 15.0 kV ETD 3.0
100.0µm
O
10/25/2012 WD Mag HV Det Spot
1:40:08 AM 20.0 mm 3068x 15.0 kV ETD 3.0
10.0µm

Discussion

Apiosordaria hamata can be recognized by its spiny ascospores in the clavate asci and is supported in the phylogeny. Its ascospores are similar in size to *A. nigeriensis* (24–32 × 20–23 µm), *A. jamaicensis* (B.M. Robison) Krug et al. (24–30 × 17–23 µm), *A. sacchari* (B.M. Robison) Krug et al. (33–40 × 25–31 µm), and *A. spinosa* (Cailleux) Krug et al. (25–32 × 18–21 µm), all of which also have spinulose to spiny ascospores (Krug et al. 1983). However, *A. hamata* is distinct in having hooked spines on the upper cell of the ascospores and in lacking germ pores, which Meléndez-Howell (1970) observed in her SEM studies of previously described *Apiosordaria* species.

Phylogenetic analysis supported the two isolates of *A. hamata* in a subclade with *A. nigeriensis, A. verruculosa,* and *Cladorrhinum* sp. (asexual morph of *Apiosordaria*) with >50% bootstrap ML value but separated from the other *Apiosordaria* species. Although *A. hamata* is morphologically similar to *A. nigeriensis*, they are separated on the ITS and LSU phylogenetic tree (Fig. 1), which clusters *A. hamata* in a monophyletic group with seven species of *Apiosordaria*. Here, morphology coupled with molecular analysis support this fungus as a new species in *Apiosordaria* (*Lasiosphaeriaceae*).

Key to species of *Apiosordaria*

1. Asci 4-spored, clavate 2
1. Asci 8-spored, clavate or cylindrical 5
2. Ascomata globose, upper ascospore cell verrucose, 18–22 × 14–17 µm; lower ascospore cell 4–6 × 5–6 µm ..... *A. effusa*
2. Ascomata pyriform, ostiolate 3
3. Upper ascospore cell spinulose or warty, 16–21 × 13–15 µm; lower ascospore cell 6–10 × 9–19 µm ..... *A. verruculosa*
3. Upper ascospore cell pitted 4
4. Lower ascospore cell 10–14 × 7.5–9 µm ..... *A. longicaudata*
4. Lower ascospore cell 4–7.5(–10) × 6–8(–11) µm ..... *A. tetraspora*
5. Ascospore wall with broad pits; ornamentation appearing as spines or irregular warts 6
5. Ascospore wall with narrow pits; ornamentation appearing as pits 18
6. Upper ascospore cell polygonal, five-angled in side view, ornamented with longitudinal ribs, 10–12 × 8–9 µm ..... *A. striatispora*
6. Upper ascospore cell more or less ellipsoidal 7
7. Ascoma ostiolate 8
7. Ascoma smooth or with another type of vestiture, non-ostiolate; ascospores overall typically larger than 14 µm 11

8. Ascoma lacking agglutinated hairs;
upper ascospore cell 10–12.5 × 6–8 µm with flat-topped spines;
lower cell 4–6 µm long; asexual morph absent ..... *A. yaeyamensis*

8. Ascoma with agglutinated hairs 9

9. Upper ascospore cell 15–19 × 10–12.5 µm; lower cell 5–6.5 µm long;
asexual morph *Chrysosporium* ..... *A. microcarpa*

9. Upper ascospore cell larger 10

10. Ascoma pyriform; upper ascospore cell pitted, 27–34 × 18–23 µm ..... *A. backusii*

10. Upper ascospore cell uniformly ornamented with numerous rounded warts,
23–28(–30) × 18–22(–23) µm; lower ascospore cell 1–5(–6) µm long;
asexual morph absent ..... *A. hispanica*

11. Ascospores with a granulose to wart-like ornamentation;
lower ascospore cell 4–5 µm long; asexual morph absent ..... *A. tuberculata*

11. Ascospores with spine-like ornamentation or flat-topped ridges;
lower ascospore cell longer 12

12. Asci cylindrical and curved; upper ascospore cell 16–20(–22) × 12–15 µm,
lower cell frequently transversely septate, 12–20 µm long;
asexual morphs *Cladorrhinum* and *Chrysosporium* ..... *A. vermicularis*

12. Asci clavate; upper ascospore cell larger; lower cell non-septate 13

13. Ascospore ornamentation 2–5.5 µm long,
upper cell (24–)25–32 × 18–21(–22) µm, lower cell 4–8 µm long;
sexual morph *Chrysosporium* (when present) ..... *A. spinosa*

13. Ascospore ornamentation shorter (≤3 µm); lower cell longer 14

14. Upper ascospore cell 33–40 × 25–31 µm; lower cell 14–20 µm long;
asexual morph absent ..... *A. sacchari*

14. Upper and lower ascospore cells shorter 15

15 Ascospore ornamentation irregular flat-topped ridges forming an incomplete
reticulum, upper cell 28.5–33 × 17–19.5 µm; lower cell ca. 8–10 µm long;
asexual morph *Chrysosporium* ..... *A. terrestris*

15. Ascospore ornamentation spine-like 16

16. Upper ascospore cell without germ pore, 26–31 × 22–26 µm, uniformly
ornamented with hooked spines of 2–3 µm diam; lower cell
sub-hyaline, conical, smooth, 12–15 µm long; asexual morph absent *A. hamata*

16. Upper ascospore cell with germ pore 17

17. Upper ascospore cell 24–32 × 20–23 µm, with a dark area surrounding
the germ pore and ornamented with spines ≤7 µm long;
lower cell 7–10 µm long ..... *A. nigeriensis*

17. Upper ascospore cell 24–30 × 17–23(–26) µm, ornamented with
spines ≤3 µm long; lower cell ca. 11–18 µm long;
asexual morph *Chrysosporium* (when present) ..... *A. jamaicensis*

18. Ascoma glabrous; ascospores overall (16–)18–21(–23) × 10–12 µm, with rugose flanges; lower ascospore cell 2.5–5 µm long; asexual morph absent ... *A. rugosa*
18. Ascoma with agglutinated hairs 19
19. Upper ascospore cell regularly pitted, 20–25 × 12–15 µm, lacking flanges; lower ascospore cell 4–5 µm long; asexual morph *Cladorrhinum* ..... *A. vestita*
19. Upper ascospore cell covered with small warts 20
20. Upper ascospore cell 27–34 × 18–23 µm, walls ornamented with shallow pits; lower ascospore cell 11–17 × 10–12 µm long ..... *A. tenuilacunata*
20. Upper ascospore cell smaller 21
21. Asexual morph *Chrysosporium*-like; upper ascospore cell 20–24 × 15–18 µm ..... *A. otanii*
21. Asexual morph absent 22
22. Upper ascospore cell 25–27(–29) × (22–)23–27 µm, uniformly ornamented with small warts; lower cell (1–) 4–6 µm long, slightly warted ..... *A. globosa*
22. Upper ascospore cell 19–24 × 8–10 µm, ornamented with small warts; lower cell 3.5–4.5 × 3–4 µm ..... *A. antarctica*

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